

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	0	rhamnose adj isomerase	US-PGPUB; USPAT; DERWENT	OR	ON	2006/11/21 12:34
L2	27	rhamnose adj isomerase	US-PGPUB; USPAT; DERWENT	OR	ON	2006/11/21 12:35
L3	2	rhamnose adj isomerase and pseudomonas adj stutzeri	US-PGPUB; USPAT; DERWENT	OR	ON	2006/11/21 12:36
L4	1	rhamnose adj isomerase and pseudomonas adj stutzeri and Izumori	US-PGPUB; USPAT; DERWENT	OR	ON	2006/11/21 12:37
L5	1	rhamnose adj isomerase and pseudomonas adj stutzeri and Takada	US-PGPUB; USPAT; DERWENT	OR	ON	2006/11/21 12:37
L6	1	rhamnose adj isomerase and pseudomonas adj stutzeri and Tokuda	US-PGPUB; USPAT; DERWENT	OR	ON	2006/11/21 12:37

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PASSWORD:

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LIFESCI, BIOTECHNO, EMBASE' AT 12:55:49 ON 21 NOV 2006
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FULL ESTIMATED COST	21.05	21.26

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CA SUBSCRIBER PRICE	-1.50	-1.50

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=> s rhamnose (w) isomerase
L2 168 RHAMNOSE (W) ISOMERASE

=> s l2 and stutzeri
L3 22 L2 AND STUTZERI

=> d ibib abs l3 1-22

L3 ANSWER 1 OF 22 MEDLINE on STN
ACCESSION NUMBER: 2006339974 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16754978
TITLE: Crystallization and preliminary X-ray diffraction studies
of L-rhamnose isomerase from
Pseudomonas stutzeri.
AUTHOR: Yoshida Hiromi; Wayoon Poonperm; Takada Goro; Izumori Ken;
Kamitori Shigehiro
CORPORATE SOURCE: Molecular Structure Research Group, Information Technology
Center and Faculty of Medicine, Kagawa University, 1750-1
Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan.
SOURCE: Acta crystallographica. Section F, Structural biology and
crystallization communications, (2006 Jun 1) Vol. 62, No.
Pt 6, pp. 550-2. Electronic Publication: 2006-05-31.
Journal code: 101226117. E-ISSN: 1744-3091.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200608
ENTRY DATE: Entered STN: 7 Jun 2006
Last Updated on STN: 5 Aug 2006
Entered Medline: 4 Aug 2006

AB L-Rhamnose isomerase from Pseudomonas stutzeri
(P. stutzeri L-RhI) catalyzes not only the reversible
isomerization of L-rhamnose to L-rhamnulose, but also isomerization
between various rare aldoses and ketoses. Purified His-tagged P.
stutzeri L-RhI was crystallized by the hanging-drop
vapour-diffusion method. The crystals belong to the monoclinic space
group P2(1), with unit-cell parameters a = 74.3, b = 104.0, c = 107.0 A,
beta = 106.8 degrees. Diffraction data have been collected to 2.0 A
resolution. The molecular weight of the purified P. stutzeri
L-RhI with a His tag at the C-terminus was confirmed to be 47.7 kDa by
MALDI-TOF mass-spectrometric analysis and the asymmetric unit is expected
to contain four molecules.

L3 ANSWER 2 OF 22 MEDLINE on STN
ACCESSION NUMBER: 2004436161 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15342115
TITLE: Novel reactions of L-rhamnose isomerase
from Pseudomonas stutzeri and its relation with
D-xylose isomerase via substrate specificity.
AUTHOR: Leang Khim; Takada Goro; Fukai Yoshinori; Morimoto Kenji;
Granstrom Tom Birger; Izumori Ken
CORPORATE SOURCE: Department of Biochemistry and Food Science, Faculty of
Agriculture and Rare Sugar Research Center, Kagawa
University, Ikenobe 2393, Miki-cho, Kagawa 761-0795, Japan.
SOURCE: Biochimica et biophysica acta, (2004 Sep 6) Vol. 1674, No.
1, pp. 68-77.

Journal code: 0217513. ISSN: 0006-3002.

PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200411
ENTRY DATE: Entered STN: 3 Sep 2004
Last Updated on STN: 2 Nov 2004
Entered Medline: 1 Nov 2004

AB Escherichia coli strain JM 109 harboring 6 x His-tag L-rhamnose isomerase (L-RhI) from Pseudomonas stutzeri allowed a 20-fold increase in the volumetric yield of soluble enzyme compared to the value for the intrinsic yield. Detailed studies on the substrate specificity of the purified His-tagged protein revealed that it catalyzed previously unknown common and rare aldo/ketotetrose, aldo/ketopentose, and aldo/ketohexose substrates in both D- and L-forms, for instance, erythrose, threose, xylose, lyxose, ribose, glucose, mannose, galactose, altrose, tagatose, sorbose, psicose, and fructose. Using a high enzyme-substrate ratio in extended reactions, the enzyme-catalyzed interconversion reactions from which two different products from one substrate were formed: L-lyxose, L-glucose, L-tagatose and D-allose were isomerized to L-xylulose and L-xylose, L-fructose and L-mannose, L-galactose and L-talose, and D-psicose and D-altrose, in that order. Kinetic studies, however, showed that L-rhamnose with Km and Vmax values of 11 mM and 240 U/mg, respectively, was the most preferred substrate, followed by L-mannose, L-lyxose, D-ribose, and D-allose. Based on the observed catalytic mode of action, these new findings reflected a hitherto undetected interrelation between L-RhI and D-xylose isomerase (D-XI).

L3 ANSWER 3 OF 22 MEDLINE on STN

ACCESSION NUMBER: 2004282507 MEDLINE

DOCUMENT NUMBER: PubMed ID: 15184124

TITLE: Cloning, nucleotide sequence, and overexpression of the L-rhamnose isomerase gene from Pseudomonas stutzeri in Escherichia coli.

AUTHOR: Leang Khim; Takada Goro; Ishimura Akihiro; Okita Masashi; Izumori Ken

CORPORATE SOURCE: Department of Biochemistry and Food Science, Faculty of Agriculture and Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa 761-0795, Japan.

SOURCE: Applied and environmental microbiology, (2004 Jun) Vol. 70, No. 6, pp. 3298-304.

Journal code: 7605801. ISSN: 0099-2240.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB121136
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 9 Jun 2004
Last Updated on STN: 11 Aug 2004
Entered Medline: 10 Aug 2004

AB The gene encoding L-rhamnose isomerase (L-RhI) from Pseudomonas stutzeri was cloned into Escherichia coli and sequenced. A sequence analysis of the DNA responsible for the L-RhI gene revealed an open reading frame of 1,290 bp coding for a protein of 430 amino acid residues with a predicted molecular mass of 46,946 Da. A comparison of the deduced amino acid sequence with sequences in relevant databases indicated that no significant homology has previously been identified. An amino acid sequence alignment, however, suggested that the residues involved in the active site of L-RhI from E. coli are conserved in that from P. stutzeri. The L-RhI gene was then overexpressed in E. coli cells under the control of the T5 promoter. The recombinant clone, E. coli JM109, produced significant levels of L-RhI activity, with

a specific activity of 140 U/mg and a volumetric yield of 20,000 U of soluble enzyme per liter of medium. This reflected a 20-fold increase in the volumetric yield compared to the value for the intrinsic yield. The recombinant L-RhI protein was purified to apparent homogeneity on the basis of three-step chromatography. The purified recombinant enzyme showed a single band with an estimated molecular weight of 42,000 in a sodium dodecyl sulfate-polyacrylamide gel. The overall enzymatic properties of the purified recombinant L-RhI protein were the same as those of the authentic one, as the optimal activity was measured at 60 degrees C within a broad pH range from 5.0 to 11.0, with an optimum at pH 9.0.

L3 ANSWER 4 OF 22 JICST-EPlus COPYRIGHT 2006 JST on STN

ACCESSION NUMBER: 1040515825 JICST-EPlus
TITLE: A Novel Enzymatic Approach to the Massproduction of
L-Galactose from L-Sorbose
AUTHOR: LEANG K; MAEKAWA K; MENAVUVU B T; MORIMOTO K; GRANSTROEM T
B; TAKADA G; IZUMORI K
CORPORATE SOURCE: Kagawa Univ., Kagawa, Jpn
SOURCE: J Biosci Bioeng, (2004) vol. 97, no. 6, pp. 383-388.
Journal Code: G0535B (Fig. 5, Tbl. 3, Ref. 37)
ISSN: 1389-1723
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
LANGUAGE: English
STATUS: New

AB Wild-type strain of *Pseudomonas cichorii* ST-24 was unable to grow on D-psicose and inductively produced D-tagatose 3-epimerase (D-TE) with D-tagatose as an inducer. We have isolated a constitutive mutant, designated strain Ka75, which had acquired a new ability to grow on a mineral salts medium containing D-psicose as a sole carbon source. The D-psicose-metabolizing mutant synthesized a high level of D-TE. When grown on the culture medium supplemented with Mn²⁺, the mutant strain produced around 250-fold higher activity than did the parent strain. Enzymatic properties of the constitutive enzyme were similar to those of the wild-type. Using the immobilized D-TE and recombinant L-rhamnose isomerase (L-RhI) from *Escherichia coli* strain JM109, a two-step enzymatic reaction was performed for massproduction of a rare aldo-hexose monosaccharide, L-galactose, from a common one, L-sorbose. In the first step, L-sorbose was epimerized to L-tagatose in a yield of 28%. The L-tagatose obtained was utilized as a starting material for L-galactose preparation by the immobilized L-RhI. At equilibrium, approximately 30% L-tagatose was isomerized to L-galactose. Finally, 7.5 g of L-galactose was obtained from 100 g of L-sorbose, viz an overall yield of 7.5%. The product obtained was purified and identified to be L-galactose by specific optical rotation and high performance liquid chromatography (HPLC) analysis, and was ultimately confirmed by ¹³C nuclear magnetic resonance (¹³C NMR) and IR spectra. (author abst.)

L3 ANSWER 5 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:434473 BIOSIS
DOCUMENT NUMBER: PREV200600423751
TITLE: Crystallization and preliminary X-ray diffraction studies
of L-rhamnose isomerase from
Pseudomonas stutzeri.
AUTHOR(S): Yoshida, Hiromi; Wayoon, Poonperm; Takada, Goro; Izumori,
Ken; Kamitori, Shigehiro [Reprint Author]
CORPORATE SOURCE: Kagawa Univ, Mol Struct Res Grp, Informat Technol Ctr,
1750-1 Ikenobe, Kagawa 7610793, Japan
kamitori@med.kagawa-u.ac.jp
SOURCE: Acta Crystallographica Section F Structural Biology and
Crystallization Communications, (JUN 2006) Vol. 62, No.
Part 6, pp. 550-552.
ISSN: 1744-3091. E-ISSN: 1744-3091.

DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Aug 2006
Last Updated on STN: 30 Aug 2006

AB L-Rhamnose isomerase from *Pseudomonas stutzeri* (P. stutzeri L-RhI) catalyzes not only the reversible isomerization of L-rhamnose to L-rhamnulose, but also isomerization between various rare aldoses and ketoses. Purified His-tagged P. stutzeri L-RhI was crystallized by the hanging-drop vapour-diffusion method. The crystals belong to the monoclinic space group P2(1), with unit-cell parameters a = 74.3, b = 104.0, c = 107.0 angstrom, beta = 106.8 degrees. Diffraction data have been collected to 2.0 angstrom resolution. The molecular weight of the purified P. stutzeri L-RhI with a His tag at the C-terminus was confirmed to be 47.7 kDa by MALDI-TOF mass-spectrometric analysis and the asymmetric unit is expected to contain four molecules.

L3 ANSWER 6 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:312786 BIOSIS

DOCUMENT NUMBER: PREV200600309538

TITLE: Large scale production of D-allose from D-psicose using continuous bioreactor and separation system.

AUTHOR(S): Morimoto, Kenji; Park, Chang-Su; Ozaki, Motofumi; Takeshita, Kei; Shimonishi, Tsuyoshi; Granstroem, Tom Birger; Takata, Goro; Tokuda, Masaaki; Izumori, Ken [Reprint Author]

CORPORATE SOURCE: Kagawa Univ, Rare Sugar Res Ctr, Kagawa 7610795, Japan izumori@ag.kagawa-u.ac.jp

SOURCE: Enzyme and Microbial Technology, (APR 1 2006) Vol. 38, No. 6, pp. 855-859.

CODEN: EMTED2. ISSN: 0141-0229.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 14 Jun 2006

Last Updated on STN: 14 Jun 2006

AB L-Rhamnose isomerase (L-RhI) from *Pseudomonas stutzeri* LL172 can convert D-psicose to D-allose. Partially purified recombinant L-RhI from *Escherichia coli* was immobilized on BCW-2510 Chitopearl beads and utilized to produce D-allose. Total 20,000 units of immobilized enzyme converted D-psicose to D-allose without remarkable decrease in the enzyme activity over 17 days. When 50% D-psicose (w/w) was applied to a column with a flow rate of 0.8 ml/min at 42 degrees C, approximately 30% D-psicose was isomerized to D-allose for 17 days. However, by reducing the flow rate to 0.4 ml/min after 17 days, D-allose was transformed at the same rate for 13 days. The total of 27 l reaction mixture was separated by simulated-Moving-Bed Chromatograph system. Approximately 2.2 l/d of 50% (w/w) reaction mixture was separated continuously. After separation, D-allose and D-psicose fractions were 31 of approximately 10% (w/w) with 95% purity and 101 of approximately 8% (w/w) with 95% purity per day, respectively. The separated D-allose solution was concentrated up to about 50% and crystallized gradually by being kept at room temperature. Crystals Of D-allose were separated from the syrup by filtration and 1.65 kg crystals of 100% purity were obtained. The D-allose crystal yield from the D-psicose substrate was approximately 10%. (c) 2005 Elsevier Inc. All rights reserved.

L3 ANSWER 7 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:461717 BIOSIS

DOCUMENT NUMBER: PREV200400464064

TITLE: Novel reactions of L-rhamnose isomerase from *Pseudomonas stutzeri* and its relation with D-xylose isomerase via substrate specificity.

AUTHOR(S): Leang, Khim; Takada, Goro; Fukai, Yoshinori; Morimoto, Kenji; Granstrom, Tom Birger; Izumori, Ken [Reprint Author]

CORPORATE SOURCE: Fac AgrDept Biochem and Food Sci, Kagawa Univ, Ikenobe
2393, Miki, Kagawa, 7610795, Japan
izumori@ag.kagawa-u.ac.jp

SOURCE: Biochimica et Biophysica Acta, (September 6 2004) Vol.
1674, No. 1, pp. 68-77. print.
ISSN: 0006-3002 (ISSN print).

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 1 Dec 2004

Last Updated on STN: 1 Dec 2004

AB Escherichia coli strain JM 109 harboring 6 x His-tag L-rhamnose isomerase (L-RhI) from Pseudomonas stutzeri allowed a 20-fold increase in the volumetric yield of soluble enzyme compared to the value for the intrinsic yield. Detailed studies on the substrate specificity of the purified His-tagged protein revealed that it catalyzed previously unknown common and rare aldo/ketotetrose, aldo/ketopentose, and aldo/ketohexose substrates in both D- and L-forms, for instance, erythrose, threose, xylose, lyxose, ribose, glucose, mannose, galactose, altrose, tagatose, sorbose, psicose, and fructose. Using a high enzyme-substrate ratio in extended reactions, the enzyme-catalyzed interconversion reactions from which two different products from one substrate were formed: L-lyxose, L-glucose, L-tagatose and D-allose were isomerized. to L-xylulose and L-xylose, L-fructose and L-mannose, L-galactose and L-talose, and D-psicose and D-altrose, in that order. Kinetic studies, however, showed that L-rhamnose with Km and Vmax values of 1.1 mM and 240 U/mg, respectively, was the most preferred substrate, followed by L-mannose, L-lyxose, D-ribose, and D-allose. Based on the observed catalytic mode of action, these new findings reflected a hitherto undetected interrelation between L-RhI and D-xylose isomerase (D-XI).
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L3 ANSWER 8 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:331372 BIOSIS

DOCUMENT NUMBER: PREV200400334105

TITLE: Cloning, nucleotide sequence, and overexpression of the L-rhamnose isomerase gene from Pseudomonas stutzeri in Escherichia coli.

AUTHOR(S): Leang, Khim; Takada, Goro; Ishimura, Akihiro; Okita, Masashi; Izumori, Ken [Reprint Author]

CORPORATE SOURCE: Fac AgrDept Biochem and Food Sci, Kagawa Univ, Miki, Kagawa, 7610795, Japan
izumori@ag.kagawa-u.ac.jp

SOURCE: Applied and Environmental Microbiology, (June 2004) Vol. 70, No. 6, pp. 3298-3304. print.
ISSN: 0099-2240 (ISSN print).

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 4 Aug 2004

Last Updated on STN: 4 Aug 2004

AB The gene encoding L-rhamnose isomerase (L-RhI) from Pseudomonas stutzeri was cloned into Escherichia coli and sequenced. A sequence analysis of the DNA responsible for the L-RhI gene revealed an open reading frame of 1,290 bp coding for a protein of 430 amino acid residues with a predicted molecular mass of 46,946 Da. A comparison of the deduced amino acid sequence with sequences in relevant databases indicated that no significant homology has previously been identified. An amino acid sequence alignment, however, suggested that the residues involved in the active site of L-RhI from E. coli are conserved in that from P. stutzeri. The L-RhI gene was then overexpressed in E. coli cells under the control of the T5 promoter. The recombinant clone, E. coli JM109, produced significant levels of L-RhI activity, with a specific activity of 140 U/mg and a volumetric yield of 20,000 U of soluble enzyme per liter of medium. This reflected a 20-fold increase in the volumetric yield compared to the value for the intrinsic yield. The

recombinant L-RhI protein was purified to apparent homogeneity on the basis of three-step chromatography. The purified recombinant enzyme showed a single band with an estimated molecular weight of 42,000 in a sodium dodecyl sulfate-polyacrylamide gel. The overall enzymatic properties of the purified recombinant L-RhI protein were the same as those of the authentic one, as the optimal activity was measured at 60degreeC within a broad pH range from 5.0 to 11.0, with an optimum at pH 9.0.

L3 ANSWER 9 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2006:916835 CAPLUS

DOCUMENT NUMBER: 145:270227

TITLE: Enzymic preparation of complex crystalline sugar comprising D-psicose and D-allose

INVENTOR(S): Izumori, Ken; Tokuda, Masaaki; Takada, Goro; Morimoto, Kenji

PATENT ASSIGNEE(S): National University Corporation Kagawa University, Japan

SOURCE: PCT Int. Appl., 33pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006093292	A1	20060908	WO 2006-JP304151	20060303
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				

PRIORITY APPLN. INFO.: JP 2005-61638 A 20050304

AB Disclosed is a process for producing a crystalline sugar comprising D-psicose and D-allose at a ratio of .apprx.1:1 to 1:4. Also disclosed is a process for producing the crystalline sugar. The solvent of the sugar solution used in the production of the complex crystalline sugar is water or a mixture of water and

ethanol. The sugar solution containing D-psicose and D-allose is produced from D-psicose with L-rhamnose isomerase to convert D-psicose into D-allose without further purification The L-rhamnose isomerase is of Pseudomonas stutzerii, or recombinant Escherichia coli.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 10 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2006:559381 CAPLUS

DOCUMENT NUMBER: 145:61502

TITLE: Purified rare saccharide mass production method using enzymic isomerization reaction and chromatography

INVENTOR(S): Kamori, Takeshi; Morimoto, Kenji; Tokuda, Masaaki; Tsusaki, Keiji; Takeshita, Kei

PATENT ASSIGNEE(S): Kagawa University, Japan; Hayashibara Biochemical Laboratories, Inc.; Fushimi Pharmaceutical Co., Ltd.

SOURCE: Jpn. Kokai Tokkyo Koho, 21 pp.

DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2006153591	A2	20060615	JP 2004-342930	20041126
PRIORITY APPLN. INFO.:			JP 2004-342930	20041126

AB A method is provided for separating only a production object rare saccharide with high purity and high recovery from a raw stock solution consisting of a mixture of rare saccharides containing a production object rare saccharide. This purified rare saccharide mass production method is characterized in that it comprises converting a substrate rare saccharide to an object rare saccharide with the function of an enzyme catalyzing an isomerization, and continuously separating an object rare saccharide fraction from the obtained raw stock solution by chromatog. with a pseudo-mobile phase. The method is applicable to producing purified D-allose as the object rare saccharide from D-psicose as the substrate rare saccharide. As the above raw stock solution utilized is a raw stock solution obtained by allowing L- rhamnose isomerase from Pseudomonas stutzeri to react with the substrate saccharide. Diagrams describing the chromatog. operation scheme are given.

L3 ANSWER 11 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:528829 CAPLUS
DOCUMENT NUMBER: 145:287413
TITLE: Crystallization and preliminary x-ray diffraction studies of L-rhamnose isomerase from Pseudomonas stutzeri
AUTHOR(S): Yoshida, Hiromi; Wayoon, Poonperm; Takada, Goro; Izumori, Ken; Kamitori, Shigehiro
CORPORATE SOURCE: Molecular Structure Research Group, Information Technology Center and Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa, 761-0793, Japan
SOURCE: Acta Crystallographica, Section F: Structural Biology and Crystallization Communications (2006), F62(6), 550-552
CODEN: ACSFCL; ISSN: 1744-3091
URL: <http://journals.iucr.org/f/issues/2006/06/00/bw5141/index.html>
PUBLISHER: Blackwell Publishing Ltd.
DOCUMENT TYPE: Journal; (online computer file)
LANGUAGE: English

AB L-Rhamnose isomerase (I) of P. stutzeri catalyzes not only the reversible isomerization of L-rhamnose to L-rhamnulose, but also the isomerization between various rare aldoses and ketoses. Here, purified His-tagged P. stutzeri I was crystallized by the hanging-drop vapor-diffusion method. The I crystals belonged to monoclinic space group P2₁, with unit-cell parameters a = 74.3, b = 104.0, c = 107.0 Å, and β = 106.8°. Diffraction data were collected to 2.0 Å resolution. The mol. weight of purified P. stutzeri I with a His tag at the C-terminus was confirmed to be 47.7 kDa by MALDI-TOF mass spectrometric anal. and the asym. unit was expected to contain 4 mols.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 12 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2006:196068 CAPLUS
 DOCUMENT NUMBER: 144:249414
 TITLE: Protein and DNA sequences of thermostable L-rhamnose isomerase gene from Bacillus and use in izumoring strategy of synthesizing rare sugars
 INVENTOR(S): Izumori, Ken; Takata, Goro; Tokuda, Masaaki
 PATENT ASSIGNEE(S): National University Corporation Kagawa University, Japan
 SOURCE: PCT Int. Appl., 48 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2006022239	A1	20060302	WO 2005-JP15236	20050823
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				

PRIORITY APPLN. INFO.: JP 2004-244253 A 20040824
 JP 2005-95538 A 20050329

AB Described are the protein and DNA sequences of thermostable L-rhamnose isomerase gene isolated from Bacillus pallidus strain 14a (FERM AP-20172) and use in izumoring strategy of synthesizing rare sugars. The L-rhamnose isomerase has the following characteristics: optimum temperature at 80°C and working temperature ranging from 30 to 80°C; optimum pH ranging from 6 to 9 and working pH ranging from 6 to 10; heat stable at up to 50°C for 1 h; enzymic activity inhibited by cobalt ion; and catalyzing the isomerization from D-psicose to D-allose. The L-rhamnose isomerase had highest activity toward L-rhamnose (relative 100%), lower activity toward L-lyxose (relative 23.9%), L-mannose (relative 11.0%), D-allose (relative 5.6%), and D-lyxose (relative 0.14%). Compared with Bacillus L-rhamnose isomerase, the L-rhamnose isomerase from Pseudomonas stutzeri strain LL172 showed lower optimum temperature (600C), less thermostable (400C), and narrower range of optimum pH (8-9).

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 13 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2006:178656 CAPLUS
 DOCUMENT NUMBER: 144:466605
 TITLE: Large scale production of D-allose from D-psicose using continuous bioreactor and separation system
 AUTHOR(S): Morimoto, Kenji; Park, Chang-Su; Ozaki, Motofumi; Takeshita, Kei; Shimonishi, Tsuyoshi; Granstroem, Tom Birger; Takata, Goro; Tokuda, Masaaki; Izumori, Ken
 CORPORATE SOURCE: Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa, 761-0795, Japan
 SOURCE: Enzyme and Microbial Technology (2006), 38(6), 855-859
 CODEN: EMTED2; ISSN: 0141-0229

PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Rhamnose isomerase (-RhI) from *Pseudomonas stutzeri* LL172 can convert D-psicose to D-allose. Partially purified recombinant L-RhI from *Escherichia coli* was immobilized on BCW-2510 Chitopearl beads and utilized to produce D-allose. Total 20,000 units of immobilized enzyme converted D-psicose to D-allose without remarkable decrease in the enzyme activity over 17 days. When 50% D-psicose (weight/weight) was applied to a column with a flow rate of 0.8 mL/min

at 42 °C, approx. 30% D-psicose was isomerized to D-allose for 17 days. However, by reducing the flow rate to 0.4 mL/min after 17 days, D-allose was transformed at the same rate for 13 days. The total of 27 L reaction mixture was separated by Simulated-Moving-Bed Chromatograph system. Approx. 2.2 l/d of 50% (weight/weight) reaction mixture was separated continuously.

After separation, D-allose and D-psicose fractions were 3 l of approx. 10% (weight/weight) with 95% purity and 10 l of approx. 8% (weight/weight) with 95% purity per day, resp. The separated D-allose solution was concentrated up to about 50% and crystallized gradually by being kept at room temperature. Crystals of D-allose were separated from the syrup by filtration and 1.65 kg crystals of 100% purity were obtained. The D-allose crystal yield from the D-psicose substrate was approx. 10%.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 14 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:900778 CAPLUS

DOCUMENT NUMBER: 141:348872

TITLE: Crystallization of D-allose in continuous enzymic manufacture of the sugar

INVENTOR(S): Kamori, Takeshi; Takada, Goro; Tokuda, Masaaki

PATENT ASSIGNEE(S): Kagawa University, Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 9 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2004298106	A2	20041028	JP 2003-95828	20030331
PRIORITY APPLN. INFO.:			JP 2003-95828	20030331

AB The D-allose manufactured from D-psicose with L-rhamnose isomerase is crystallized with alcs. such as ethanol and methanol. Because the method does not require the removal of buffer solns., the D-allose can be continuously manufactured and purified with high-purity using immobilized enzyme or microorganism such as *Pseudomonas stutzeri*. Manufacture and purification of D-allose from D-psicose with immobilized L-rhamnose isomerase of *P. stutzerii* was shown. Also given was a working procotol of ethanol precipitation and crystallization of the D-allose from the reaction mixture

L3 ANSWER 15 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:730590 CAPLUS

DOCUMENT NUMBER: 141:390897

TITLE: Novel reactions of L-rhamnose isomerase from *Pseudomonas stutzeri*

and its relation with D-xylose isomerase via substrate specificity

AUTHOR(S): Leang, Khim; Takada, Goro; Fukai, Yoshinori; Morimoto, Kenji; Granstrom, Tom Birger; Izumori, Ken

CORPORATE SOURCE: Department of Biochemistry and Food Science, Faculty of Agriculture and Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa, 761-0795, Japan

SOURCE: Biochimica et Biophysica Acta, General Subjects (2004), 1674(1), 68-77
CODEN: BBGSB3; ISSN: 0304-4165

PUBLISHER: Elsevier B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Escherichia coli strain JM 109 harboring 6+ His-tag L-rhamnose isomerase (L-RhI) from Pseudomonas stutzeri allowed a 20-fold increase in the volumetric yield of soluble enzyme compared to the value for the intrinsic yield. Detailed studies on the substrate specificity of the purified His-tagged protein revealed that it catalyzed previously unknown common and rare aldo/ketotetrose, aldo/ketopentose, and aldo/ketohexose substrates in both D- and L-forms, for instance, erythrose, threose, xylose, lyxose, ribose, glucose, mannose, galactose, altrose, tagatose, sorbose, psicose, and fructose. Using a high enzyme-substrate ratio in extended reactions, the enzyme-catalyzed interconversion reactions from which two different products from one substrate were formed: L-lyxose, L-glucose, L-tagatose and D-allose were isomerized to L-xylulose and L-xylose, L-fructose and L-mannose, L-galactose and L-talose, and D-psicose and D-altrose, in that order. Kinetic studies, however, showed that L-rhamnose with Km and Vmax values of 11 mM and 240 U/mg, resp., was the most preferred substrate, followed by L-mannose, L-lyxose, D-ribose, and D-allose. Based on the observed catalytic mode of action, these new findings reflected a hitherto undetected interrelation between L-RhI and d-xylose isomerase (D-XI).

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 16 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:606548 CAPLUS

DOCUMENT NUMBER: 141:136202

TITLE: Cloning of L-rhamnose isomerase gene from Pseudomonas stutzeri and use in Izumoring strategy of synthesizing rare sugars

INVENTOR(S): Izumori, Ken; Takada, Goro; Tokuda, Masaaki

PATENT ASSIGNEE(S): Japan Represented by President of Kagawa University, Japan

SOURCE: PCT Int. Appl., 65 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004063369	A1	20040729	WO 2004-JP131	20040109
W:			AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ	
JP 2005102503	A2	20050421	JP 2003-299371	20030822
EP 1589102	A1	20051026	EP 2004-701121	20040109
R:			AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK	
US 2006148061	A1	20060706	US 2006-541822	20060213
PRIORITY APPLN. INFO.:			JP 2003-5041	A 20030110

JP 2003-96046	A	20030331
JP 2003-299371	A	20030822
JP 2003-96043	A	20030331
WO 2004-JP131	W	20040109

AB L-rhamnose isomerase originating in *Pseudomonas stutzerii*, encoding gene, recombinant expression, and use in synthesis of rare sugars, are disclosed. In the Izumoring (Fig. 1) strategy of synthesizing rare sugars, a reaction system for producing rare sugars of many types is established by acquiring an isomerase which acts on various rare aldoses and, therefore, is most efficient in producing various rare ketoses. The gene encoding L-rhamnose isomerase (L-RhI) from *Pseudomonas stutzeri* was cloned into *Escherichia coli* and sequenced. A sequence anal. of the DNA responsible for the L-RhI gene revealed an open reading frame of 1,290 bp coding for a protein of 430 amino acid residues with a predicted mol. mass of 46,946 Da. A comparison of the deduced amino acid sequence with sequences in relevant databases indicated that no significant homol. has previously been identified. An amino acid sequence alignment, however, suggested that the residues involved in the active site of L-RhI from *E. coli* are conserved in that from *P. stutzeri*. The L-RhI gene was then overexpressed in *E. coli* cells under the control of the T5 promoter. The recombinant clone, *E. coli* JM109, produced significant levels of L-RhI activity, with a specific activity of 140 U/mg and a volumetric yield of 20,000 U of soluble enzyme per L of medium. The purified recombinant enzyme showed a single band with an estimated mol. weight of 42,000 in a sodium dodecyl sulfate-polyacrylamide gel. The overall enzymic properties of the purified recombinant L-RhI protein were the same as those of the authentic one, as the optimal activity was measured at 60°C within a broad pH range from 5.0 to 11.0, with an optimum at pH 9.0. Production of D-lyxose from D-glucose via D-arabitol and D-xylulose and of L-erythrose from erythritol via L-erythrulose using L-ribose isomerase, is described.

L3 ANSWER 17 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:514141 CAPLUS

DOCUMENT NUMBER: 141:202223

TITLE: Cloning, nucleotide sequence, and overexpression of the L-rhamnose isomerase gene from *Pseudomonas stutzeri* in *Escherichia coli*

AUTHOR(S): Leang, Khim; Takada, Goro; Ishimura, Akihiro; Okita, Masashi; Izumori, Ken

CORPORATE SOURCE: Department of Biochemistry and Food Science, Faculty of Agriculture and Rare Sugar Research Center, Kagawa University, Miki, 761-0795, Japan

SOURCE: Applied and Environmental Microbiology (2004), 70(6), 3298-3304

CODEN: AEMIDF; ISSN: 0099-2240

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The gene encoding L-rhamnose isomerase (L-RhI) from *Pseudomonas stutzeri* was cloned into *Escherichia coli* and sequenced. A sequence anal. of the DNA responsible for the L-RhI gene revealed an open reading frame of 1290 bp coding for a protein of 430 amino acid residues with a predicted mol. mass of 46,946 Da. A comparison of the deduced amino acid sequence with sequences in relevant databases indicated that no significant homol. has previously been identified. An amino acid sequence alignment, however, suggested that the residues involved in the active site of L-RhI from *E. coli* are conserved in that from *P. stutzeri*. The L-RhI gene was then overexpressed in *E. coli* cells under the control of the T5 promoter. The recombinant clone, *E. coli* JM109, produced significant levels of L-RhI activity, with a specific activity of 140 U/mg and a volumetric yield of 20,000 U of soluble enzyme per L of medium. This reflected a 20-fold increase in the volumetric yield compared to the value for the intrinsic yield. The

recombinant L-RhI protein was purified to apparent homogeneity on the basis of three-step chromatog. The purified recombinant enzyme showed a single band with an estimated mol. weight of 42,000 in a SDS-polyacrylamide gel.

The overall enzymic properties of the purified recombinant L-RhI protein were the same as those of the authentic one, as the optimal activity was measured at 60° within a broad pH range from 5.0 to 11.0, with an optimum at pH 9.0.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 18 OF 22 LIFESCI COPYRIGHT 2006 CSA on STN

ACCESSION NUMBER: 2006:42790 LIFESCI

TITLE: Large scale production of d-allose from d-psicose using continuous bioreactor and separation system

AUTHOR: Morimoto, Kenji; Park, Chang-Su; Ozaki, Motofumi; Takeshita, Kei; Shimonishi, Tsuyoshi; Granstroem, Tom Birger; Takata, Goro; Tokuda, Masaaki; Izumori, Ken

CORPORATE SOURCE: Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa 761-0795, Japan; E-mail: izumori@ag.kagawa-u.ac.jp

SOURCE: Enzyme and Microbial Technology [Enzyme Microb. Technol.], (20060401) vol. 38, no. 6, pp. 855-859.
ISSN: 0141-0229.

DOCUMENT TYPE: Journal

FILE SEGMENT: W2; A

LANGUAGE: English

SUMMARY LANGUAGE: English

AB 1-Rhamnose isomerase (1-RhI) from *Pseudomonas stutzeri* LL172 can convert d-psicose to d-allose. Partially purified recombinant 1-RhI from *Escherichia coli* was immobilized on BCW-2510 Chitopearl beads and utilized to produce d-allose. Total 20,000 units of immobilized enzyme converted d-psicose to d-allose without remarkable decrease in the enzyme activity over 17 days. When 50% d-psicose (w/w) was applied to a column with a flow rate of 0.8 ml/min at 42 degree C, approximately 30% d-psicose was isomerized to d-allose for 17 days. However, by reducing the flow rate to 0.4 ml/min after 17 days, d-allose was transformed at the same rate for 13 days. The total of 27 l reaction mixture was separated by Simulated-Moving-Bed Chromatograph system. Approximately 2.2 l/d of 50% (w/w) reaction mixture was separated continuously. After separation, d-allose and d-psicose fractions were 3 l of approximately 10% (w/w) with 95% purity and 10 l of approximately 8% (w/w) with 95% purity per day, respectively. The separated d-allose solution was concentrated up to about 50% and crystallized gradually by being kept at room temperature. Crystals of d-allose were separated from the syrup by filtration and 1.65 kg crystals of 100% purity were obtained. The d-allose crystal yield from the d-psicose substrate was approximately 10%.

L3 ANSWER 19 OF 22 LIFESCI COPYRIGHT 2006 CSA on STN

ACCESSION NUMBER: 2006:12155 LIFESCI

TITLE: Cloning, Nucleotide Sequence, and Overexpression of the L-Rhamnose Isomerase Gene from *Pseudomonas stutzeri* in *Escherichia coli*

AUTHOR: Leang, K.; Takada, G.; Ishimura, A.; Okita, M.; Izumori, K.

CORPORATE SOURCE: Department of Biochemistry and Food Science, Faculty of Agriculture and Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa 761-0795, Japan; E-mail: izumori@ag.kagawa-u.ac.jp

SOURCE: Applied and Environmental Microbiology [Appl. Environ. Microbiol.], (20040600) vol. 70, no. 6, pp. 3298-3304.
ISSN: 0099-2240.

DOCUMENT TYPE: Journal

FILE SEGMENT: J

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The gene encoding L-rhamnose isomerase (L-RhI) from *Pseudomonas stutzeri* was cloned into *Escherichia coli* and sequenced. A sequence analysis of the DNA responsible for the L-RhI gene revealed an open reading frame of 1,290 bp coding for a protein of 430 amino acid residues with a predicted molecular mass of 46,946 Da. A comparison of the deduced amino acid sequence with sequences in relevant databases indicated that no significant homology has previously been identified. An amino acid sequence alignment, however, suggested that the residues involved in the active site of L-RhI from *E. coli* are conserved in that from *P. stutzeri*. The L-RhI gene was then overexpressed in *E. coli* cells under the control of the T5 promoter. The recombinant clone, *E. coli* JM109, produced significant levels of L-RhI activity, with a specific activity of 140 U/mg and a volumetric yield of 20,000 U of soluble enzyme per liter of medium. This reflected a 20-fold increase in the volumetric yield compared to the value for the intrinsic yield. The recombinant L-RhI protein was purified to apparent homogeneity on the basis of three-step chromatography. The purified recombinant enzyme showed a single band with an estimated molecular weight of 42,000 in a sodium dodecyl sulfate-polyacrylamide gel. The overall enzymatic properties of the purified recombinant L-RhI protein were the same as those of the authentic one, as the optimal activity was measured at 60 degree C within a broad pH range from 5.0 to 11.0, with an optimum at pH 9.0.

L3 ANSWER 20 OF 22 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2006085957 EMBASE
TITLE: Large scale production of D-allose from D-psicose using continuous bioreactor and separation system.
AUTHOR: Morimoto K.; Park C.-S.; Ozaki M.; Takeshita K.; Shimonishi T.; Granstrom T.B.; Takata G.; Tokuda M.; Izumori K.
CORPORATE SOURCE: K. Izumori, Rare Sugar Research Center, Kagawa University, Miki-cho, Kagawa 761-0795, Japan. izumori@ag.kagawa-u.ac.jp
SOURCE: Enzyme and Microbial Technology, (1 Apr 2006) Vol. 38, No. 6, pp. 855-859. .
Refs: 14
ISSN: 0141-0229 CODEN: EMTED2
PUBLISHER IDENT.: S 0141-0229(05)00367-4
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 16 Mar 2006
Last Updated on STN: 16 Mar 2006

AB L-Rhamnose isomerase (l-RhI) from *Pseudomonas stutzeri* LL172 can convert d-psicose to d-allose. Partially purified recombinant l-RhI from *Escherichia coli* was immobilized on BCW-2510 Chitopearl beads and utilized to produce d-allose. Total 20,000 units of immobilized enzyme converted d-psicose to d-allose without remarkable decrease in the enzyme activity over 17 days. When 50% d-psicose (w/w) was applied to a column with a flow rate of 0.8 ml/min at 42°C, approximately 30% d-psicose was isomerized to d-allose for 17 days. However, by reducing the flow rate to 0.4 ml/min after 17 days, d-allose was transformed at the same rate for 13 days. The total of 27 l reaction mixture was separated by Simulated-Moving-Bed Chromatograph system. Approximately 2.2 l/d of 50% (w/w) reaction mixture was separated continuously. After separation, d-allose and d-psicose fractions were 3 l of approximately 10% (w/w) with 95% purity and 10 l of approximately 8% (w/w) with 95% purity per day, respectively. The separated d-allose solution was concentrated up to about 50% and crystallized gradually by being kept at room temperature. Crystals of d-allose were separated from the syrup by filtration and 1.65 kg crystals of 100% purity were obtained. The d-allose crystal yield from the d-psicose substrate was approximately

L3 ANSWER 21 OF 22 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2004376615 EMBASE
TITLE: Novel reactions of L-rhamnose isomerase
from *Pseudomonas stutzeri* and its relation with
D-xylose isomerase via substrate specificity.
AUTHOR: Leang K.; Takada G.; Fukai Y.; Morimoto K.; Granstrom T.B.;
Izumori K.
CORPORATE SOURCE: K. Izumori, Dept. of Biochem. and Food Science, Fac. Agric.
and Rare Sugar Res. Ctr., Kagawa Univ., Ikenobe 2393, M.,
Kagawa, Japan. izumori@ag.kagawa-u.ac.jp
SOURCE: Biochimica et Biophysica Acta - General Subjects, (6 Sep
2004) Vol. 1674, No. 1, pp. 68-77. .
Refs: 51
ISSN: 0304-4165 CODEN: BBGSB3
PUBLISHER IDENT.: S 0304-4165(04)00137-0
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 24 Sep 2004
Last Updated on STN: 24 Sep 2004

AB *Escherichia coli* strain JM 109 harboring 6x His-tag L-rhamnose
isomerase (L-RhI) from *Pseudomonas stutzeri* allowed a
20-fold increase in the volumetric yield of soluble enzyme compared to the
value for the intrinsic yield. Detailed studies on the substrate
specificity of the purified His-tagged protein revealed that it catalyzed
previously unknown common and rare aldo/ketotetrose, aldo/ketopentose, and
aldo/ketohexose substrates in both D- and L-forms, for instance,
erythrose, threose, xylose, lyxose, ribose, glucose, mannose, galactose,
altrose, tagatose, sorbose, psicose, and fructose. Using a high
enzyme-substrate ratio in extended reactions, the enzyme-catalyzed
interconversion reactions from which two different products from one
substrate were formed: L-lyxose, L-glucose, L-tagatose and D-allose were
isomerized to L-xylulose and L-xylose, L-fructose and L-mannose,
L-galactose and L-talose, and D-psicose and D-altrose, in that order.
Kinetic studies, however, showed that L-rhamnose with K(m) and V(max)
values of 11 mM and 240 U/mg, respectively, was the most preferred
substrate, followed by L-mannose, L-lyxose, D-ribose, and D-allose. Based
on the observed catalytic mode of action, these new findings reflected a
hitherto undetected interrelation between L-RhI and D-xylose isomerase
(D-XI). .COPYRGT. 2004 Elsevier B.V. All rights reserved.

L3 ANSWER 22 OF 22 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2004249900 EMBASE
TITLE: Cloning, nucleotide sequence, and overexpression of the L-
rhamnose isomerase gene from *Pseudomonas*
stutzeri in *Escherichia coli*.
AUTHOR: Leang K.; Takada G.; Ishimura A.; Okita M.; Izumori K.
CORPORATE SOURCE: K. Izumori, Dept. of Biochem. and Food Science, Fac. of
Agric./Rare Sugar Res. Ctr., Kagawa University, Miki-cho,
Kagawa 761-0795, Japan. izumori@ag.kagawa-u.ac.jp
SOURCE: Applied and Environmental Microbiology, (2004) Vol. 70, No.
6, pp. 3298-3304. .
Refs: 36
ISSN: 0099-2240 CODEN: AEMIDF
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 9 Jul 2004

Last Updated on STN: 9 Jul 2004

AB The gene encoding L-rhamnose isomerase (L-RhI) from *Pseudomonas stutzeri* was cloned into *Escherichia coli* and sequenced. A sequence analysis of the DNA responsible for the L-RhI gene revealed an open reading frame of 1,290 bp coding for a protein of 430 amino acid residues with a predicted molecular mass of 46,946 Da. A comparison of the deduced amino acid sequence with sequences in relevant databases indicated that no significant homology has previously been identified. An amino acid sequence alignment, however, suggested that the residues involved in the active site of L-RhI from *E. coli* are conserved in that from *P. stutzeri*. The L-RhI gene was then overexpressed in *E. coli* cells under the control of the T5 promoter. The recombinant clone, *E. coli* JM109, produced significant levels of L-RhI activity, with a specific activity of 140 U/mg and a volumetric yield of 20,000 U of soluble enzyme per liter of medium. This reflected a 20-fold increase in the volumetric yield compared to the value for the intrinsic yield. The recombinant L-RhI protein was purified to apparent homogeneity on the basis of three-step chromatography. The purified recombinant enzyme showed a single band with an estimated molecular weight of 42,000 in a sodium dodecyl sulfate-polyacrylamide gel. The overall enzymatic properties of the purified recombinant L-RhI protein were the same as those of the authentic one, as the optimal activity was measured at 60°C within a broad pH range from 5.0 to 11.0, with an optimum at pH 9.0.